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Speed versus stability – structure-activity effects on the assembly of two-component gels

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This paper reports the structural modification of a two-component gelation system comprising a 1:1 complex formed between a peptide carboxylic acid and phenylethylamine. Changing amino acids has a profound effect on the speed of gel formation and the minimum gelation concentration (MGC) yet the thermal stability of the gel remains unchanged. Variable temperature NMR studies demonstrate that at room temperature, the speed at which the gel forms is controlled by the solubility of the acid-amine complexes, which mediates the initial nucleation step required for gel assembly. On increasing the temperature, however, a thermodynamic enthalpy-entropy balance means all of the gels break down at around the same temperature. Those gels which are more favourably and rapidly formed at room temperature on enthalpic grounds are also more temperature sensitive as a consequence of the greater entropic cost of efficient packing within the gel fibres. This constitutes a rare example in which the time required for gelation can be structurally controlled, with NMR providing unique insight into the dynamics of these gel-phase materials. We suggest that in the future, combining solvent and solute (gelator) solubility parameters may provide further insight into these materials.

Introduction

Gels are colloidal soft matter systems in which a solid-like nanoscale network spans a liquid-like phase.[1] Low molecular weight gels in which small molecules assemble into nanoscale fibres constituting a solid-like network have been known for well over 100 years, and are used in a number of industrial applications as well as having many potential high-tech uses as a consequence of their highly tunable molecular structures.[2]

In general, there have been relatively few studies of self-assembling systems focussing on the speed of gelation.[3] It is known that in some supramolecular gels instant gelation can occur,[4] while in other cases a stimulus is required in order to trigger gelation – such as heating/cooling, or ultrasound.[5] The kinetics of gelation can be followed in detail using spectroscopic techniques and fitted to Avrami type kinetic behaviour or fractal growth mechanisms to provide insight into the dimensionality of fibre growth.[6] Importantly, there has been some debate over the role of solvent and solvation in controlling gelation kinetics, and it has been suggested that Hansen solubility parameters can be used to predict gelation rate.[7] In elegant work, Meijer and co-workers used VT-CD methods to understand the kinetics of nanofibre formation in a self-assembling system and demonstrated that solvent played an intimate role.[8] It is also worth noting that gels are kinetically trapped materials and can change structure and evolve on standing.[3f,9] Given the importance of the speed of gel assembly in many applications – from *in vivo* gelation for tissue engineering[10] to gelation in fuel oils for oil spillage remediation,[11] and the ability of gelation speed to optimise/modify gel morphology and performance,[12] it is surprising that there have been so few attempts to gain structural control over this feature.

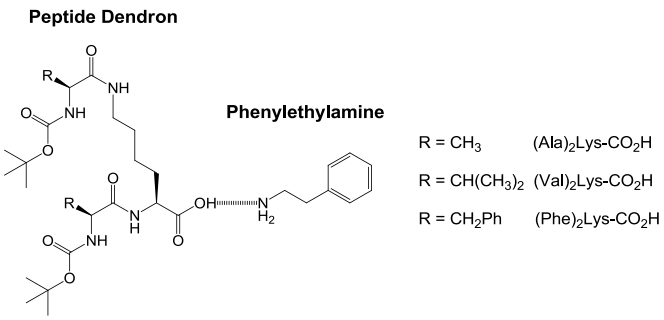

Figure 1. Structure of dendritic peptide:phenylethylamine complexes investigated in this paper.

In recent years, multi-component gels, in which two compounds must form a complex in order for gelation to occur have emerged at the forefront of self-assembled gels.[13] This approach to gelation has the advantage of being uniquely tunable – the presence of two components and solvent significantly increases the scope for structural variation and by tuning one or both components, which can have very different impacts on the molecular recognition pathways which underpin

self-assembly. We have been working with a two-component organogel based on a dendritic peptide acid based on lysine which interacts with an amine (e.g. Fig. 1).[14] The complexes assemble into nanofibres and sample-spanning gels as a consequence of intermolecular hydrogen bond interactions between the peptides, while the presence of the amine helps control the overall solubility of the complex in such a way as to encourage aggregation of the system from the solvent into 'solid-like' gel nanofibres in the solvent of choice – consistent with the view that gelation is often considered to be analogous to a precipitation process, only driven by controlled non-covalent interactions in a single dimension. An important feature is that in some cases, gelation is instant and occurs simply on mixing the two components.[3f,15] This led us to become interested in what controlled the speed of gelation under ambient conditions, and the dynamics of these materials. We report here a simple yet relatively rare approach to tuning the speed of gelation via a structure-activity relationship study. Modifying the structure of the gelator complex changes its solubility and hence its ability to nucleate gel fibres. However, once these gels have formed an enthalpy/entropy balance ensures that they all have very similar thermal stabilities.

Results and Discussion

Synthesis of Gelators

The new peptide gelators studied here are based on our previously investigated dendritic $(Lys)_2Lys\text{-}CO_2H$ structure,[3f,14,15] but with the two peripheral amino acids being changed from lysine to other amino acids – specifically alanine (Ala), valine (Val) or phenylalanine (Phe) (Fig. 1). These amino acids do not possess hydrogen bonding groups on their side chains, so each peptide has exactly the same pattern of hydrogen bonding (CONH) groups responsible for gel formation. However, these systems differ significantly in terms of steric bulk and hydrophobicity of the amino acid side chain, in the order Ala<Val<Phe. As the amine component, we selected phenylethylamine – in part because this was the optimal amine for gelation with the lysine-derived peptide,[3f] but also because this structure is a pharmacophore for neurotransmitter drugs, and understanding gelation with this structure may allow us to optimise drug sensing or controlled release gel systems. The desired peptides were synthesised via a standard three-step procedure: (i) protecting L-lysine as its methyl ester, (ii) coupling with the relevant Boc-protected amino acid in a TBTU-mediated reaction, and (iii) base-mediated ester hydrolysis to unveil the free acid. All products were synthesised in good yield and characterised using all appropriate spectroscopic techniques (see Supp. Info.).

Initial Gelation Studies

Initial gelation studies focussed on using the peptide and amine in toluene with concentrations of 25 mM for each component. Under these conditions, simple mixing led to gelation. Interestingly, however, there were significant differences in gelation speed between the three peptides (Table 1). $(Ala)_2Lys\text{-}CO_2H$ formed a gel instantly on mixing the two components, $(Val)_2Lys\text{-}CO_2H$ formed a gel on standing for ca. 1-5 minutes but $(Phe)_2Lys\text{-}CO_2H$ only formed a gel on standing for longer periods of time (e.g. >30 min). On lowering the concentration of both components to 15 mM, $(Ala)_2Lys\text{-}CO_2H$ still formed an instant gel unlike the other gels. At even lower concentrations $(Ala)_2Lys\text{-}CO_2H$ formed a gel with a short induction time (10 s at 7.5 mmol and 1-2 min at 2.5 mmol). Clearly, the peripheral amino acid has profound effects on gelation speed. We decided that it was of great value to try and gain an enhanced understanding of this process in terms of the dynamics of these self-assembled materials.

Table 1. Gelation rates with gelators investigated in this study in toluene – all gelators were mixed with phenylethylamine in a 1:1 ratio.

Peptide Dendron	Concentration / mM	Gelation Time
$(Phe)_2Lys\text{-}CO_2H$	25	>30 min
$(Val)_2Lys\text{-}CO_2H$	25	1-5 min
$(Ala)_2Lys\text{-}CO_2H$	25	Instant
$(Ala)_2Lys\text{-}CO_2H$	15	Instant
$(Ala)_2Lys\text{-}CO_2H$	7.5	10 s
$(Ala)_2Lys\text{-}CO_2H$	2.5	1-2 min

Gel Studies – Thermal Stability (T_{gel} Measurement)

We anticipated that because $(Ala)_2Lys\text{-}CO_2H$ formed gels more rapidly than $(Val)_2Lys\text{-}CO_2H$ and $(Phe)_2Lys\text{-}CO_2H$, it may also mean its gels are more stable. To probe this, we monitored thermal stabilities using simple and reproducible tube inversion methodology in standard tubes at a fixed heating rate of 1ºC/min.[16] It should be noted that when gels form too rapidly, they are sometimes inhomogeneous as the rate of gelation becomes competitive with the rate of mixing – to study the stability of these gels in further detail, all gels were made in homogeneous form by applying a heat-cool cycle. Surprisingly, at 25 mM, even though under ambient conditions the gels form at very different rates, all of the gels had near identical thermal stabilities of ca. 77ºC (Fig. 2). Clearly this appears at first glance to be somewhat conterintuitive.

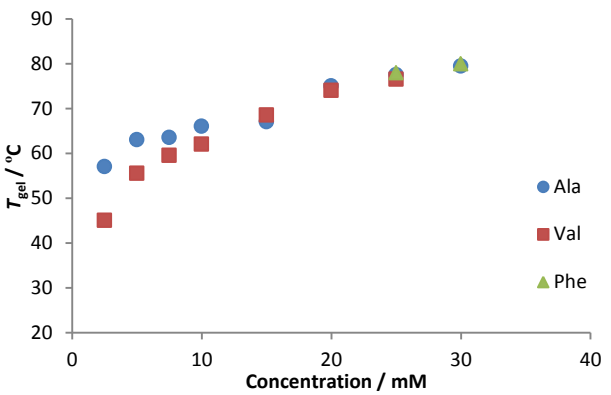

Figure 2. Variation of thermal stability of gels in toluene with concentration of the acid-amine complex.

Probing the variation of thermal stability with concentration (Fig. 2), it is clear that $(Ala)_2Lys\text{-}CO_2H$ and $(Val)_2Lys\text{-}CO_2H$

are more effective gelation systems at lower concentrations. They are able to form gels at much lower concentrations than $(Phe)_2Lys\text{-}CO_2H$. The minimum gelation concentration (MGC) for $(Ala)_2Lys\text{-}CO_2H$ and $(Val)_2Lys\text{-}CO_2H$ is <2.5 mM, while for $(Phe)_2Lys\text{-}CO_2H$ it is 25 mM. Furthermore, at concentrations below 10 mM the thermal stability of $(Ala)_2Lys\text{-}CO_2H$ > $(Val)_2Lys\text{-}CO_2H$. It is therefore clear that in the low concentration regime, gel formation and indeed thermal stability reflects the speed of gelation with the alanine gelator forming the most stable gel, as well as assembling the most quickly.

These macroscopic studies therefore clearly indicated differences in behaviour at ambient conditions, or low concentration, compared to the behaviour at elevated concentrations and temperatures (see Fig. 3). In order to understand these differences, we needed to probe what was happening in the gels on a molecular level, in dynamic terms.

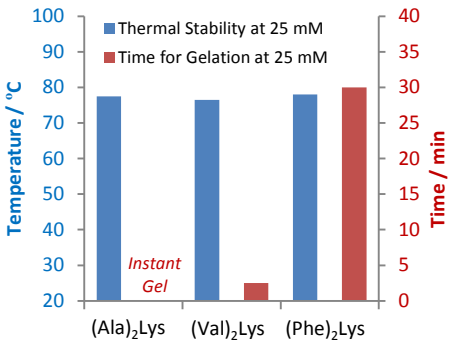

Figure 3. Summary of experimental results (all performed on 25 mM samples) indicating the significant effect of structural change on time for gelation (red), but its lack of impact on the thermal stability of the gel network (blue).

Two Component Gels – NMR Spectroscopy Studies

NMR methods have previously proved extremely powerful in understanding the dynamics within gels.[17,18a] Mobile 'liquid-like' molecules can be observed by NMR, while molecules incorporated within the 'solid-like' gel network are NMR-invisible. The use of a mobile internal standard (in the case diphenylmethane) then allows direct quantification of the numbers of mobile molecules within the gel sample.

We used NMR to confirm the stoichiometry of the complex responsible for gelation. By 'titrating' the amine into the peptide acid, we can determine how much of each is immobilised within the fibres, and hence find the point at which a stoichiometric balance is achieved. In each case, a 1:1 stoichiometry gave rise to equivalent amounts of each component being immobilised (see Fig. 4 for an example and Supp Info). Furthermore, the amount of mobile amine significantly increases once all of the peptide acid has been bound. We can therefore confidently assign gelation as dependent on the formation of a 1:1 acid:amine complex. We would note that the presence of excess amine causes slightly more peptide acid to become immobilised, presumably as it pushes the equilibrium further towards the 1:1 complex and self-assembly into nanofibres. It should also be noted that under 1:1 conditions not all of the molecules are immobilised – this reflects the dynamic nature of these gels, the formation of which depends on gelator solubility and nucleation, which is discussed in more detail below.

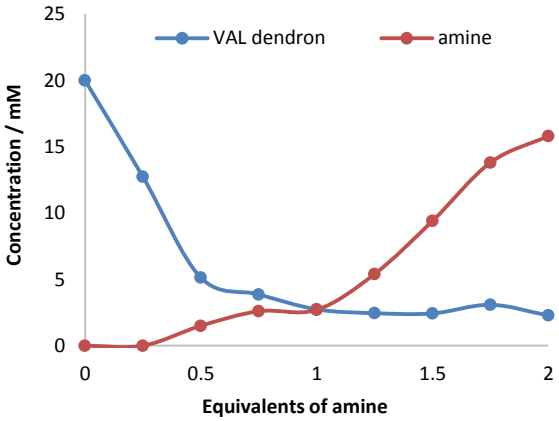

Figure 4. Titration of phenylethylamine into $(Val)_2Lys\text{-}CO_2H$ (20 mM) with the amount of mobile peptide dendron and amine being determined by NMR spectroscopy at 25ºC.

In this system, gelation occurs through a multi-step process: (i) formation of an acid:amine complex, (ii) nucleation of the acid-amine complexes into a proto-fibril, (iii) chain elongation, and (iv) fibre-fibre interactions to form a sample-spanning network capable of supporting a gel.[18] In the sections below, we attempt to probe the factors which control this assembly process in more detail.

Interactions and Dynamics within Gels – Variable Temperature NMR Studies

To further understand how the gel network forms for each of the different gelation systems, we employed variable temperature (VT) NMR studies. We initially took samples based on $(Phe)_2Lys\text{-}CO_2H$ and phenylethylamine at 20 mM and heated them while monitoring the NMR spectra (Fig. 5). The fact that $(Phe)_2Lys\text{-}CO_2H$ does not form a gel very well is reflected in the relatively high concentration of this gelator in the mobile phase under ambient conditions – i.e. the gelator is relatively soluble, and hence highly mobile. Indeed, at room temperature, ca. 5.5 mM of dendron is mobile, indicating that only the remaining 14.5 mM is assembled into a solid-like network – ca. 70%. As the temperature is increased from 20ºC to 80ºC, the amount of mobile gelator increases, with the largest increase between 60ºC and 80ºC, in agreement with the macroscopic T_{gel} value observed for a fully formed $(Phe)_2Lys\text{-}CO_2H$ network.

The relatively high solubility at room temperature would suggest that this gelator is struggling to nucleate and assemble under these conditions (see further discussion below). This may explain why $(Phe)_2Lys\text{-}CO_2H$ does not actually form a full sample-spanning gel under these conditions, and even at 25 mM can only form a gel relatively slowly. This would be consistent with a model in which $(Phe_2)Lys\text{-}CO_2H$ is simply too

soluble in these solvent conditions to aggregate well. As such, we suggest that the high level of dynamic behaviour at the molecular level caused by the solubility of the peptide, as evidenced by this NMR technique, limits the ability to nucleate gel fibres and slows the macroscopically observed gelation rate. The large change in mobility at 60-80ºC suggests that those molecules which do actually manage to assemble, form chain-extended self-assembled fibres which breakdown at this temperature, even though there are not sufficient of them, or they do not interact with one another well enough to form a full self-assembled gel.

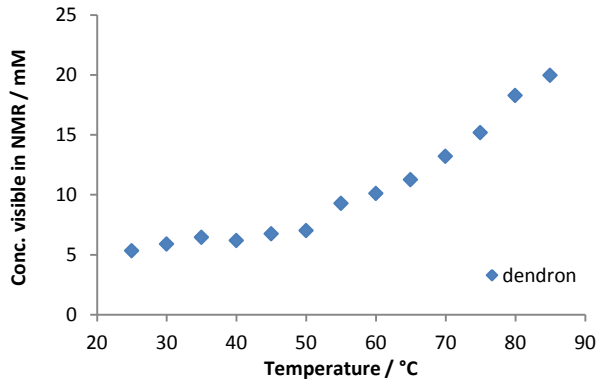

Figure 5. Mobility of $(Phe)_2Lys\text{-}CO_2H$ in the presence of phenylethylamine (both 20 mM) as assessed by NMR methods with variation of temperature.

We repeated this study with $(Val_2)Lys\text{-}CO_2H$ (Fig. 6). $(Val_2)Lys\text{-}CO_2H$ has considerably less solubility/mobility in this NMR study. Indeed, <3 mM of dendron or amine are mobile under ambient conditions – i.e. >85% is immobilised in the solid-like fibres. This gelator is therefore better able to nucleate into solid-like fibres at room temperature – in agreement with its faster gelation as observed macroscopically. On increasing the temperature, the gelator becomes increasingly mobile up to 70ºC. Interestingly, this temperature is little different to that observed for $Phe_2Lys\text{-}CO_2H$ – in agreement with the macroscopically observed T_{gel} value.

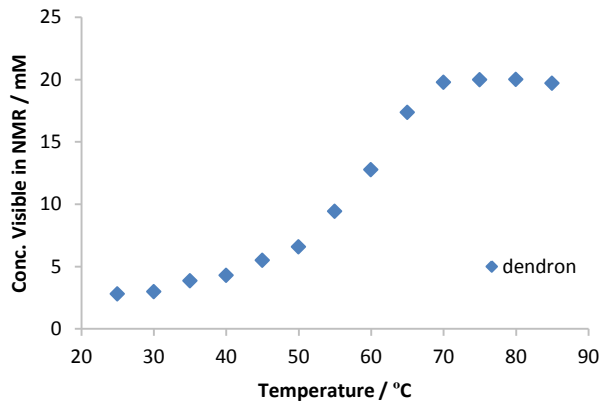

Figure 6. Mobility of $(Val)_2Lys\text{-}CO_2H$ in the presence of phenylethylamine (both 20 mM) as assessed by NMR methods with variation of temperature.

For $(Ala)_2Lys\text{-}CO_2H$, the study had to be performed at a lower concentration of 10 mM owing to its exceptional gel forming ability which made it difficult to form samples in NMR tubes. This already reflects the greater potential of this compound to assemble solid-like fibres. However, even though the concentration was lower for this compound, which should reduce self-assembly, a far greater proportion of the molecules was actually being immobilised at room temperature. Indeed, <0.05 mM of the peptide was mobile (i.e. >95% immobilised, Fig. 7). This agrees with the greater stability of the $(Ala)_2Lys\text{-}CO_2H$ gel network under low concentration conditions. We suggest that the lower solubility of this system than $(Phe)_2Lys\text{-}CO_2H$ or $(Val)_2Lys\text{-}CO_2H$ means it can better nucleate and cross-link gel fibres, directly correlating with its instant gelation. Intriguingly, on heating, the solid-like gel fibres disassemble at a similar temperature to the previously studied gelators (60-80ºC), in agreement with macroscopic T_{gel} studies.

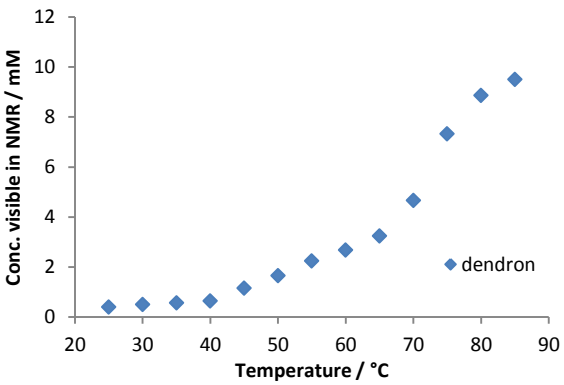

Figure 7. Mobility of $(Ala)_2Lys\text{-}CO_2H$ in the presence of phenylethylamine (both 10 mM) as assessed by NMR methods with variation of temperature.

In summary, this study suggests that differences in the speed of gelation, and differences in gelation ability at low concentration, are associated with differences in the dynamics at the molecular scale, associated with the solubility of the gelator, which programs its ability to nucleate gel fibres. It is logical that the phenylalanine based gelator is more soluble in toluene, than the other gelators. Indeed, the solubility in apolar toluene will inversely correlate with hydrophobicity of the amino acids. This conclusion that solubility has a key impact o the speed of gelation is in agreement with other recent studies which have shown that gelation kinetics can correlate with solubility parameters.[7b] Interestingly, in this previous work, the solvent was varied in order to determine the effects of solubility, whereas in this study, structural changes to the gelator appear to be having an equivalent effect. Our NMR approach, because it probes molecular dynamics within solid-like gels, is a very powerful method for providing experimental insight into the origins of these effects.

The solubility data obtained from these VT NMR experiments can also be used to calculate thermodynamic parameters, ΔH_{diss} and ΔS_{diss}, for the gel-sol transition (Table 2). This is achieved using a van't Hoff treatment of the data, assuming that these thermodynamic parameters do not vary with temperature and that the gel-sol transition is analogous to the dissolution of a solid – as reported previously and outlined

in more detail in the ESI.[18] Clearly, the gel-sol transition is more of a multi-step process than a first order phase transition – as such, the derived thermodynamic parameters refer to the processes involved in the overall gel-sol transition, which will be some combination of nucleation, fibre growth and fibre-fibre interactions. Nonetheless, these thermodynamic data are highly revealing, especially in comparison of related families of gelators, and they help explain the intriguing similar thermal stabilities of these gels even though they are very different under ambient conditions.

For $(Phe)_2Lys\text{-}CO_2H$, the enthalpy and entropy are both relatively small – suggestive of a network which has a low enthalpy and entropy of formation (i.e. the gel network is not particularly stabilised and is relatively disordered). Conversely, $(Ala)_2Lys\text{-}CO_2H$ has a much higher enthalpy and entropy of formation and clearly forms a much more favoured and ordered network. As might be expected, ΔG_{diss} at RT / kJ mol^{-1}lies somewhat intermediate between these two extremes. In all cases there is an enthalpy-entropy balance,[19] with the more enthalpically favoured networks (larger ΔH_{diss}) also being better packed and more ordered (larger ΔS_{diss}).

Table 2. Thermodynamic data for the gel-sol transition as determined by van't Hoff analysis of VT NMR data.

Peptide	ΔH_{diss} / kJ mol^{-1}	ΔS_{diss} / J $K^{-1}mol^{-1}$
$(Phe)_2Lys\text{-}CO_2H$	27.7	44.8
$(Val)_2Lys\text{-}CO_2H$	48.1	108
$(Ala)_2Lys\text{-}CO_2H$	56.6	121

To truly understand the impact of ΔH_{diss} and ΔS_{diss} on the whole gelation event, we can then convert them into free energy values (ΔG_{diss}) using the relationship $\Delta G_{diss} = \Delta H_{diss} - T\Delta S_{diss}$ (Table 3). We can do this at room temperature (T = 25ºC) to gain insight into the thermodynamic driving force for gelation under ambient conditions.

Table 3. Analysis of free energy data using the thermodynamic parameters for the gel-sol transition from Table 1 and % solubilities of each of the dendrons as determined from the VT NMR experiments.

Peptide	ΔG_{diss} at 298 K / kJ mol^{-1}	% Solubility at 298 K
$(Phe)_2Lys\text{-}CO_2H$	14.3	27.0%
$(Val)_2Lys\text{-}CO_2H$	15.9	13.9%
$(Ala)_2Lys\text{-}CO_2H$	20.5	4.0%

As is evident, at room temperature, $(Ala)_2Lys\text{-}CO_2H$ has a much greater thermodynamic driving force for gelation than $(Phe)_2Lys\text{-}CO_2H$, with $(Val)_2Lys\text{-}CO_2H$ being intermediate. This is in agreement with the experimental observations, which showed that $(Ala)_2Lys\text{-}CO_2H$ had by far the lowest solubility at room temperature and was mainly assembled within the solid-like fibre network – unlike $(Phe)_2Lys\text{-}CO_2H$, which had relatively high solubility under these conditions and therefore could not support a very effective gel. We present the percentage solubilities of the dendrons under ambient conditions, as determined by NMR, in Table 3. This correlates with the observed minimum gelation concentration and the speed of gelation, and supports our proposal that favoured nucleation of the gel fibres enhances the rate of gelation under these conditions.

On heating the gel samples up from room temperature to measure the thermal stability, however, the entropy term will become increasingly dominant (because $\Delta G_{diss} = \Delta H_{diss} - T\Delta S_{diss}$). The relatively high entropy of dissociation for $(Ala)_2Lys\text{-}CO_2H$ explains why even though this gel forms the most thermodynamically favoured gels at room temperature, as the temperature increases, this gel becomes destabilised more significantly than those with lower entropies. Therefore, although at room temperature the gels formed by these peptides have very different potentials for gelation, as the temperature increases, the ΔG_{diss} values become increasingly similar as a consequence of the entropy term. This thermodynamic analysis helps explain why at higher concentrations, these gels all exhibit similar T_{gel} values. The very ordered, solid-like fibres assembled by $(Ala)_2Lys\text{-}CO_2H$ (or $(Val)_2Lys\text{-}CO_2H$) and phenylethylamine which drive gelation event under ambient conditions are ultimately, on heating, also their downfall, leading to less thermal stability than might have been expected as a consequence of the high entropic cost of assembly.

It is worth noting that the ΔG_{diss} values in Table 3 indicate that all of these compounds have a driving force for nucleation – it is therefore worth considering why at ambient conditions, $(Val)_2Lys\text{-}CO_2H$ only assembles slowly (20 mM) after input of thermal energy, and $(Phe)_2Lys\text{-}CO_2H$ (20 mM) does not fully assemble into a gel. Clearly, there is an energy barrier to gelation which has to be overcome. Classical nucleation theory[20] would indicate that the rate of nucleation decreases exponentially with the size of this energy barrier – as such, it has a significant influence. According to nucleation theory, the energy barrier is associated with the favourable difference in bulk free energies between the gel fibre and bulk solvent and the free energy penalty of the growing gel fibre surface. We suggest that the lower solubility of $(Ala)_2Lys\text{-}CO_2H$ under ambient conditions (Table 3) reflects the fact that this compound is best able to overcome the nucleation energy barrier, leading to the rapid onset of its gelation.

Clearly these observations are in agreement with other landmark studies which have correlated gelation speed with solubility parameters.[6,7b] However, in those studies where solvent is varied, the existence of defined parameters for each solvent enables a quantitative correlation. In this case, differently to previous work, it is the gelator structure that is modifying the solubility in a given solvent. The quantifiable solubility parameters (associated with the solvent) are therefore the same in each case. As such, differences in gelation speed reflect the differences in gelator structure/solubility, which are much harder to parameterise. Notably, when we attempted to use these two-component systems for the gelation of different solvents (details not reported here), we found different rates of gelation – presumably associated with differences in the relative solubility of each gelation system in each new solvent system. Furthermore, in a two-component system such as this, each individual component, and the complex they form, will

each have a distinctive solubility profile. As such, this system is harder to analyse than our previous one-component systems.[18a] At this point, we suggest that experimental, empirical use of NMR to determine solubility under ambient conditions and/or at variable concentration, for any given gelator/solvent combination gives a highly effective insight into its potential for gelation. We intend to perform more wide-ranging studies across a broader range of two-component systems in order to develop a more quantitative and predictive understanding. We suggest that combining computationally-derived solute parameters (such as the Abraham parameters)[21] to describe the gelator (solute), with solvent parameters (such as the Hansen parameters) to describe the solvent,[6,7b] is the best way forwards for fully understanding gelation speed and stability – but this was beyond the scope of this preliminary study.

Conclusions

In conclusion, in these new two-component gels, the peptide structure directs the speed of gelation and the minimum concentration at which the gels can form. However, in the concentrated regime, the fully-formed gel networks have similar thermal stabilities. NMR evidence suggests that assembly and fibre nucleation depend intimately on the solubility of the complex, which is strongly influenced by the peptide. This is in agreement with other landmark studies on rate of gelation which have mainly focussed on variation of solvent (rather than gelator structure).[6,7b] Interestingly, the enthalpy/entropy balance means that although the gel formed by $(Ala)_2Lys\text{-}CO_2H$ is more effectively packed and thermodynamically favoured at room temperature, as the temperature rises, a greater entropic term than the other gels means it is destabilised to a greater extent. We propose that for this reason, the fully formed gels exhibit similar T_{gel} values.

This is one of the first examples in which the speed of gelation can be easily tuned in a structural way – with solvent-gelator interactions in control. Given the importance of gelation onset speed in a wide range of applications,[2] we suggest that attempts to tune and optimise gelation kinetics in a predictive manner are of significance. In particular, rapid *in situ* self-assembly and gel formation is highly desirable for applications of gels, for example, in environmental science[10] or *in vivo*.[11]

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Notes and references

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† The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Electronic Supplementary Information (ESI) available: Synthesis and characterisation of all novel compounds, data from NMR stoichiometry studies and thermodynamic analysis of VT NMR studies. See DOI: 10.1039/b000000x/

1 (*a*) *Molecular Gels: Materials with Self-Assembled Fibrillar Networks*, ed. P. Terech and R. G. Weiss, Springer, Dordrecht, Netherlands, 2006. (*b*) J. H. van Esch, *Langmuir*, 2009, **25**, 8392-8394. (*c*) J. W. Steed, *Chem. Commun.*, 2011, **47**, 1379-1383. (*d*) R. G. Weiss, *J. Am. Chem. Soc.*, 2014, **136**, 7519-7530.

2 (*a*) A. R. Hirst, B. Escuder, J. F. Miravet and D. K. Smith, *Angew. Chem. Int. Ed.*, 2008, **47**, 8002-8018. (*b*) S. Banerjee, R. K. Das and U. Maitra, *J. Mater. Chem.*, 2009, **19**, 6649-6687. (*c*) A. Dawn, T. Shiraki, S. Haraguchi, S.-i. Tamaru and S. Shinkai, *Chem. Asian J.*, 2011, **6**, 266-282.

3 (*a*) M. Suzuki, Y. Nakajima, M. Yumoto, M. Kimura, H. Shirai and K. Hanabusa, *Org. Biomol. Chem.*, 2004, **2**, 1155-1159. (*b*) D. R. Trivedi and P. Dastidar, *Chem. Mater.*, 2006, **18**, 1470-1478. (*c*) D. G. Velazquez, D. D. Diaz, A. G. Ravelo and J. J. M. Tellado, *J. Am. Chem. Soc.*, 2008, **130**, 7967-7973. (*d*) R. K. Das, D. R. Trivedi, N. N. Adarsh and P. Dastidar, *J. Org. Chem.*, 2009, **74**, 7111-7121. (*e*) P. Sahoo, D. K. Kumar, S. R. Raghavan and P. Dastidar, *Chem. Asian J.*, 2011, **6**, 1038-1047. (*f*) M. Fontanillo, C. A. Angulo-Pachon, B. Escuder and J. F. Miravet, *J. Coll. Interface Sci.*, 2013, **412**, 65-71. (*g*) W. Edwards and D. K. Smith, *J. Am. Chem. Soc.*, 2013, **135**, 5911-5920.

4 J. W. P. Schmelzer, *Kinetics of Nucleation, Aggregation and Ageing* in *Molecular Gels, Materials with Self-Assembled Fibrillar Networks*, Eds. R. G. Weiss and P. Terech, Springer, Dordrecht, 2006, Chapter 8.

5 X. Yu, L. Chen, M. Zhang and T. Yi, *Chem. Soc. Rev.*, 2014, **43**, 5346-5371.

6 (*a*) X. Huang, P. Terech, S. R. Raghavan and R. G. Weiss, *J. Am. Chem. Soc.,* 2005, **127**, 4336-4344. (*b*) X. Huang, S. R. Raghavan, P. Terech, R. G. Weiss, *J. Am. Chem. Soc.*, 2006, **128**, 15341-15352. (*c*) D. J. Cornwell, B. O. Okesola and D. K. Smith, D. K. *Soft Matter*, 2013, **9**, 8730-8736. (*d*) H. Takeno, T. Mochizuki, *Coll. Polym. Sci.*, 2013, **291**, 2783-2789. (*e*) Y. Liu, R.-Y. Yao, J.-L. Li, B. Yuan, M. Han, P. Wang and X.-Y. Liu, *Cryst. Eng. Commun.*, 2014, **16**, 5402-5408. (*f*) D. J. Cornwell, B. O. Okesola and D. K. Smith, *Angew. Chem. Int. Ed.*, 2014, **53**, 12461-12465.

7 (*a*) M. A. Rogers, A. G. Marangoni, *Langmuir*, 2009, **25**, 8556-8566. (*b*) K. K. Fiehn, H. Oh, R. Hashemipour, R. G. Weiss, S. R. Raghavan, *Soft Matter*, 2014, **10**, 2632-2640.

8 (*a*) P. Jonkheijm, P. van der Schoot, A. P. H. J. Schenning and E. W. Meijer, *Science*, 2006, **313**, 80-83. (*b*) S. Cantekin, Y. Nakano, J. C. Everts, P. van der Schoot, E. W. Meijer and A. R. A. Palmans, *Chem. Commun.*, 2012, **48**, 3803-3805.

9 (*a*) P. Terech, N. M. Sangeetha and U. Maitra, *J. Phys. Chem. B*, 2006, **110**, 15224-15233. (*b*) J. R. Moffat and D. K. Smith, *Chem. Commun*., 2008, 2248-2250. (*c*) P. Terech, *Langmuir*, 2009, **25**, 8370-8372. (*d*) J. Cui, A. Liu, Y. Guan, J. Zheng, Z. Shen and X. Wan, *Langmuir*, 2010, **26**, 3615-3622. (*e*) B. Roy, A. Saha, A. Esterrani and A. K. Nandi, *Soft Matter*, 2010, **6**, 3337-3345. (*f*) S. Debnath, S. Roy and R. V. Ulijn, *J. Am. Chem. Soc.*, 2013, **135**, 16789-16792. (*g*) P. Zhu, X. Yan, Y. Su, Y. Yang, and J. Li, *Chem.*

Eur. J., 2010, **16**, 3176-3183. (*h*) M. M. Smith and D. K. Smith, *Soft Matter*, 2011, **7**, 4856-4860. (*i*) J. Boekhoven, J. M. Poolman, C Maity, F. Li, L. van der Mee, C. B. Minkenberg, E. Mendes, J. H. van Esch and R. Eelkema, *Nature Chem.*, 2013, **5**, 433-437.

10 (*a*) J. H. Collier, J. S. Rudra, J. Z. Gasiorowski and J. P. Jung, *Chem. Soc. Rev.*, 2010, **39**, 3413-3424. (*b*) D. M. Ryan and B. L. Nilsson, *Polym. Chem.*, 2012, **3**, 18-33.

11 (*a*) S. Bhattacharya and Y. Krishnan-Ghosh, *Chem. Commun.*, 2001, 185-186. (*b*) S. R. Jadhav, P. K. Vemula, R. Kumar, S. R. Raghavan and G. John, *Angew. Chem. Int. Ed.*, 2010, **49**, 7695-7698. (*c*) C.-C. Tsai, Y.-T. Cheng, L.-C. Shen, K.-C. Chang, I.-T. Ho, J.-H. Chu and W.-S. Chung, *Org. Lett.*, 2013, **15**, 5830-5833.

12 (*a*) J.-L. Li and X.-Y. Liu, *J. Phys. Chem. B*, 2009, **113**, 15467-15472. (*b*) P. Xue, R. Lu, X. Yang, L. Zhao, D. Xu, Y. Liu, H. Zhang, H. Nomoto, M. Takafuji and H. Ihara, *Chem. Eur. J.*, 2009, **15**, 9824-9835. (*c*) I. A. Coates and D. K. Smith, *Chem. Eur. J.*, 2009, **15**, 6340-6344. (*d*) J.-L. Li and X.-Y. Liu, *Adv. Funct. Mater.*, 2010, **20**, 3196-3216. (*e*) J.-L. Li, B. Yuan, X.-Y. Liu, X.-G. Wang and R.-Y. Wang, *Cryst. Growth Des.*, 2011, **11**, 3227-3234. (f) R.-Y. Wang, P. Wang, J.-L. Li, B. Yuan, Y. Liu, L. Li and X.-Y. Liu, *Phys. Chem. Chem. Phys.*, 2013, **15**, 3313-3319. (*g*) V. J. Nebot, S. Diaz-Oltra, J. Smets, S. Fernandez-Prieto, J. F. Miravet and B. Escuder, *Chem. Eur. J.*, 2014, **20**, 5762-5767.

13 (*a*) A. R. Hirst and D. K. Smith, *Chem. Eur. J.*, 2005, **11**, 5496-5508. (*b*) L. E. Buerkle and S. J. Rowan, *Chem. Soc. Rev.*, 2012, **41**, 6089-6102. (*c*)

14 (*a*) A. R. Hirst, D. K. Smith, M. C. Feiters and H. P. M. Geurts, *J. Am. Chem. Soc.*, 2003, **125**, 9010-9011. (*b*) A. R. Hirst, D. K. Smith, M. C. Feiters and H. P. M. Geurts, *Langmuir*, 2004, **20**, 7070-7077. (*c*) A. R. Hirst and D. K. Smith, *Org. Biomol. Chem.*, 2004, **2**, 2965-2971. (*d*) B. Huang, A. R. Hirst, D. K. Smith, V. Castelletto and I. W. Hamley, *J. Am. Chem. Soc.*, 2005, **127**, 7130-7139. (*e*) A. R. Hirst, D. K. Smith and J. P. Harrington, *Chem. Eur. J.*, 2005, **11**, 6552-6559. (*f*) J. R. Hardy, A. R. Hirst and D. K. Smith, *Soft Matter*, 2012, **8**, 3399-3406.13.

15 W. Edwards and D. K. Smith, *J. Am. Chem. Soc.*, 2014, **136**, 1116-1124.

16 S. R. Raghavan and B. H. Cipriano, *Gel Formation: Phase Diagrams using Tabletop Rheology and Calorimetry* in *Molecular Gels, Materials with Self-Assembled Fibrillar Networks*, Eds. R. G. Weiss and P. Terech, Springer, Dordrecht, 2006, Chapter 8.

17 (*a*) J. Makarević, M. Jokić, V. Perić, V. Tomasić, B. Kojić-Prodić and M. Žinić, *Chem. Eur. J.*, 2001, **15**, 3328-3341. (*b*) Y. E. Shapiro, *Progr. Polym. Sci.*, 2011, **36**, 1184-1253. (*c*) B. Escuder, M. LLusar and J. F. Miravet, *J. Org. Chem.*, 2006, **71**, 7747-7752. (*d*) V. J. Nebot, J. Armengol, J. Smets, S. F. Prieto, B. Escuder and J. F. Miravet, *Chem. Eur. J.*, 2012, **18**, 4063-4072.

18 (*a*) A. R. Hirst, I. A. Coates, T. R. Boucheteau, J. F. Miravet, B. Escuder, V. Castelletto, I. W. Hamley and D. K. Smith, *J. Am. Chem. Soc.*, 2008, **130**, 9113-9121. (*b*) F. Aparicio, F. Garcia and L. Sanchez, *Chem. Eur. J.*, 2013, **19**, 3239-3248. (*c*) B. J. Cafferty, I. Gallego, M. C. Chen, K. I. Farley, R. Eritja and N. V. Hud, *J. Am. Chem. Soc.*, 2013, **135**, 2447-2450.

19 (*a*) J. D. Dunitz, *Chem and Biol.*, 1995, **2**, 709-712. (*b*) L. Liu and Q. X. Guo, *Chem. Rev.*, 2001, **101**, 673-695.

20 (*a*) D. W. Oxtoby, *Acc. Chem. Res.* 1998, **31**, 91-97. (*b*) R. P. Sear, *J. Phys. Condens. Matter* 2007, **19**, 133101.

21 (*a*) M. H. Abraham and J. Le, *J. Pharm. Sci.* 1999, **88**, 868-880. (*b*) J. Jover, R. Bosque and J. Sales, *J. Chem. Inf. Comput. Sci.* 2004, **44**, 1098-1106.

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Speed versus stability – structure-activity effects on the assembly of two-component gels

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Modifying the peripheral peptides dramatically changes the time required for gelation under ambient conditions within the self-assembled fibres, whilst an enthalpy-entropy balance means that as the temperature increases, the thermal stability of the gels remains very similar indeed.